Supplementary Information

From Molecular to Macroscopic: The Rational Design of a Self-Assembled 3D DNA Crystal

Jianping Zheng,[1‡] Jens J. Birktoft,[1‡] Yi Chen,[2‡] Tong Wang,[1] Ruojie Sha,[1] Pamela E. Constantinou,[1§] Stephan L. Ginell[3], Chengde Mao,[2]* & Nadrian C. Seeman[1]*

[1]Department of Chemistry, New York University, New York, NY 10003, USA,

[2]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA,

[3]Structural Biology Center, Argonne National Laboratory, Argonne, IL 60439, USA

Email: jz437@nyu.edu; jens.knold@gmail.com; chen4@purdue.edu; tw343@nyu.edu; ruojie.sha@nyu.edu; pcp4124@rice.edu; ginell@anl.gov; mao@purdue.edu; ned.seeman@nyu.edu

[‡]These authors contributed equally.

[§]Current Address: Dept. of Bioengineering, Rice University, 6100 Main Street, MS-142, Houston, TX 77005, USA

***Corresponding Authors:** Address all correspondence to:

Nadrian C. Seeman
Department of Chemistry
New York University
New York, NY 10003
212-998-8395 (t)
212-260-7905 (f)
ned.seeman@nyu.edu

Chengde Mao
Department of Chemistry
Purdue University
West Lafayette, IN
765-494-0498 (t)
765-494-0239 (f)
mao@purdue.edu

X-Ray Diffraction Data Processing

Data Collection and Structure Solution: Crystals were treated and data were collected as summarized in the main text. Diffraction images collected at beamlines X6A and X25 at the National Synchrotron Light Source, Brookhaven National Laboratory were processed using HKL-2000;[1] those collected at beamline 19ID at the Structural Biology Center, Advanced Photon Source, using an ADSC Q315 detector, were processed using HKL3000.[2] The nick and the missing phosphate group in the central strand (blue in Figure 1) in principle destroy the 3-fold rotational symmetry of the triangle; however, the identity of the three sticky end pairs is expected to restore this symmetry, placing the nick in three different sites. This averaging is borne out in the data processing of the data at 4 Å resolution: Processing the diffraction data in either of space group P1 or space group R3 results in essentially the same merging statistics. Processing in R3 provides a higher level of redundancy, so this option was chosen for all subsequent steps. The resulting data were fed into *hkl2map*,[3] a graphic interface for the *Shelxc/d/e* programs.[4,5] The SAD (Single Anomalous Dispersion) signal enabled *Shelxd* to identify 4 out of the 4 possible unique iodine sites in the asymmetric unit. *Shelxe* assigned the hand and produced a set of phases, providing an initial, experimental map in which nucleic acid/solvent boundaries and the major and minor groves of DNA could be resolved. A model of DNA based on standard B-DNA parameters were generated in *COOT*[6] and iodine atoms were added to the respective nucleotides. The resulting model was fitted into the SAD-derived electron density maps by matching the predicted iodine positions with their matching density peaks in the experimental electron density map. The crossover junctions were modeled by using the Holliday junction structure by Ho and coworkers [7] (PDB code 1DCW) as a template. The angle between the two "legs" was stepwise increased from ~ 40° to ~ 70°, so as to maximize the match between model and electron density obtained from the SAD phasing. The change was done in steps of about 5° and the structures were energy minimized at each

interval using the program *MOLOC*.[8] The resulting model was subsequently refined against the data of the iodo-derivative using the *PHENIX* program package,[9] followed by refinement against the native data set including all observed data extending to 4.0 Å. Data-collection and processing statistics are listed in Table S1. Figures 2 & 3 of the main text were generated using *COOT*[6] and *CHIMERA*.[10]

Table S1. Experimental Data

Data Collection, and processing:		
Crystal Type	Native [1a]	Iodinated Derivative [1b]
Unit Cell (R3 – Rhombohedral setting)	a=69.22 Å, α =101.44^o	a=68.85 Å, α =102.17^o
Content of ASU[2]/ unit cell	one triangle per ASU and per unit cell	
Unit Cell (H3 - Hexagonal setting)	a=b=107.161 Å, c=93.144 Å α =β=90.0^o, γ=120.0^o	a=b=107.141 Å, c=90.676 α =β=90.0^o, γ=120.0^o
Content of ASU/ unit cell	one third triangle per ASU; three triangles per unit cell	
Resolution (Å)	17.3 – 4.01 Å	35.0-5.10 Å
Wavelength (Å)	1.0 Å	1.7 Å
Redundancy [3]	7.3 (6.0)	5.9 (2.9)
Completeness (%)	96.1 (100.0)	92.7 (70.5)
R_{merge} [4]	0.067 (0.594)	0.110 (0.331)
I/σ(I)	10.9 (0.9)	7.9 (2.3)
Refinement Statistics:		
Resolution (Å)	17.3 – 4.01 Å	
$R_{working}$ / R_{free}	0.240 / 0.309	
Number of nucleic acid atoms	854	
Number of non-nucleic acid atoms	0	
R.m.s.d. from ideal bond length / bond angle	0.016 Å / 1.364^o	

[1a] Data collected at beamline 19ID at APS
[1b] Data collected at beamline X25 at NSLS
[2] ASU = asymmetric unit
[3] Numbers in parenthesis represent values for the highest resolution bin.
[4] $R_{merge} = \Sigma(I_{obs} - I_{avg})^2 / \Sigma(I_{avg})^2$

REFERENCES

1. Otwinowski, Z. & Minor, W., Processing of X-ray diffraction data collected in oscillation mode, *Methods Enzymol.* **276**, 307-326 (1997).

2. Minor, W., Cymborowski, M., Otwinowski, Z. & Chruszez, M. (2006). HKL-3000: the integration of the data reduction and structure solution – from diffraction images to an initial model in minutes. *Acta Cryst*. **D62**, 859-866 (2006).

3. Pape, T. & Schneider, T. R. HKL2MAP: A graphical user interface for macromolecular phasing with SHELX programs, *J. Appl. Cryst*. **37**, 843–844 (2004).

4. Sheldrick G.M. Macromolecular phasing with SHELXE, *Z. Krist.* **217**, 644–650 (2002).

5. Uson I., Sheldrick G. (1999) Advances in direct methods for protein crystallography. *Curr. Opinion Struct. Biol.* 9: 643–648.

6. Emsley P, Cowtan K (2004) Coot: model-building tools for molecular graphics, *Acta Cryst.* **D60**: 2126-2132.

7. Eichmann, B.F., Vargason, J.M., Mooers, B.H.M., Ho, P.S., The Holliday junction in an inverted repeat DNA sequence: Sequence effects on the structure of four-way junctions, *Proc. Nat. Acad. Sci. (USA)* **97**, 3971-3976 (2000).

8. Gerber P.R. (1998), Charge distribution from a simple molecular orbital type calculation and non-bonding interaction terms in the force field MAB, *J. Comput. Aided Mol. Design* **12**, 37-51.

9. P.D. Adams, R.W. Grosse-Kunstleve, L.-W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter and T.C. Terwilliger (2002) PHENIX: building new software for automated crystallographic structure determination *Acta Cryst.* **D58**, 1948-1954

10. Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. "UCSF Chimera - A Visualization System for Exploratory Research and Analysis." *J. Comput. Chem.* **25,1605**-1612 (2004).